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FACTORS AFFECTING STEAM STERILIZATION OF SOME ALBERTA SOILS

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April, 1946.

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FACTORS AFFECTING STEAM STERILIZATION

OF SOME ALBERTA SOILS

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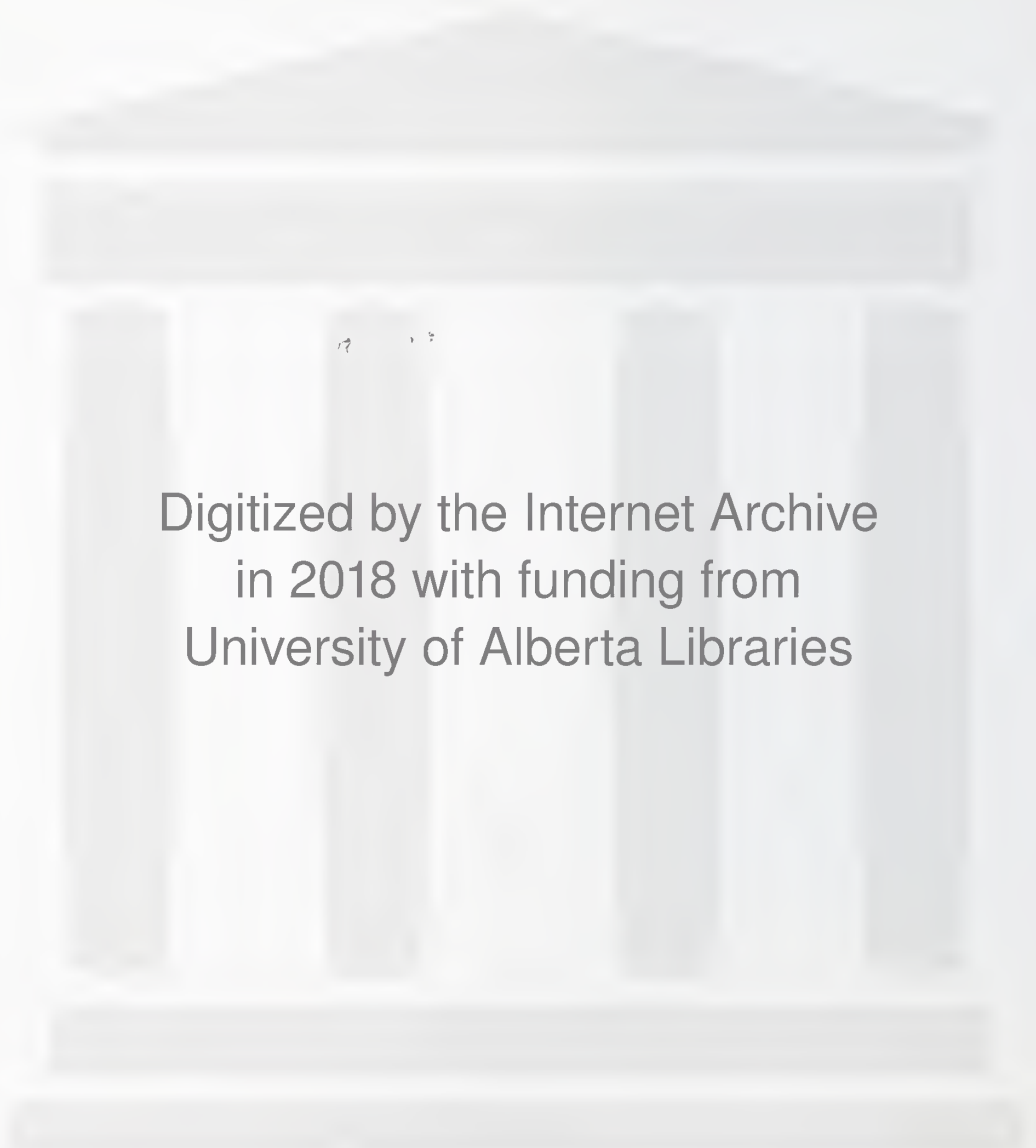
submitted to the University of Alberta in
partial fulfilment of the requirements for
the degree of Master of Science

UNDER THE DIRECTION OF

DR. J. D. NEWTON

Edmonton, Canada

April, 1946.



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INTRODUCTION

It would be of great advantage if the soil microbiologist could study singly and in artificial grouping the microflora of the soil in their natural medium. On the surface the problem appears a simple one of killing all the organisms in the soil, and then recontaminating as desired. It is in this seemingly simple process of sterilization that the complexity of the problem arises.

The present available methods of achieving soil sterility are the application of moist or dry heat, and chemical disinfectants. As a result of their application, the chemical and physical nature of the soil has been shown to be changed to such an extent that the purpose, which is to obtain media conditions closely related with actual field conditions, appears in a measure defeated. Nevertheless, heat sterilized soil has been frequently used to advantage in studying soil organisms, especially in pathology.

A review of the literature indicates that with dry heat the amount of soluble matter in the soil increases gradually with increasing temperature until 200°C (7, 15) is reached. At this temperature dry heat causes a measurable

loss of ammonia from the soil. At higher temperatures chemical losses and changes are greatly intensified. Physical changes induced by dry heat appear to be at a minimum below that temperature but at a maximum above it. Unfortunately, most of the literature available for review was confined to the application of dry heat, and a clear picture of the effect of moist heat was not obtainable.

It was thus reasoned that if a soil could be sterilized at a temperature below the seemingly critical temperature of 200°C , the resultant chemical and physical changes might not be great enough to preclude the possibility of using sterile soil as a natural medium for the study of the microflora. Apart from this, information on the time and temperature required to sterilize soil would have practical application in laboratory work. With this in mind an investigation into the minimum time, temperature, and optimum soil condition for achieving sterility was made. Because of its high efficiency as a sterilizing agent, steam heat was used in this study, despite the suggestion that it would intensify the chemical and physical changes.

REVIEW OF THE LITERATURE

The published literature concerning the practice of sterilizing materials by chemical and physical methods has been adequately reviewed by McCulloch (16) and Rahn (25).

The application of these methods to the sterilization of soil has been found to induce certain changes in the soil's chemical and physical constitution. Recognizing this, Coleman, Lint, and Kopeloff (5) conducted an investigation to find the most efficient method of sterilizing a soil with the least resulting chemical and physical changes. They compared intermittent sterilization of moist and dry clay loam by dry heat at 82°C with the intermittent application of various chemicals in vacuum, under pressure, and in combination with heat. In no case did they achieve sterility, but it is interesting to note that the application of dry heat to moist soil was much more efficient and resulted in less chemical change for the same degree of destruction than any of the other methods. For comparative purposes they autoclaved a fifty gram sample for 15 minutes at 15 pounds pressure (121°C) and achieved sterility, but with an increase in water soluble solids eight times greater than that obtained with dry heat on moist soil.

The effect of sterilizing and disinfecting a soil by means of the application of heat and chemicals has been the subject of numerous investigations. Kopeloff and Coleman (15) in their review of the work prior to 1916 report that many of

the early workers found an increase in both the organic and inorganic water soluble matter in heat treated soils. Furthermore, the amount of increase in soluble matter appeared to vary directly with the amount of organic matter. This led some workers to propose steaming as a means of measuring the fertility of a soil. The theory was that the more easily and largely the organic matter was decomposed by steaming under pressure, the more easily and extensively the plant-food of the soil became available to the plants. This was supported by other workers who found that sterilized loam soil produced good crops while sterilized subsoil produced poor crops. Additional support came from the observation that the steaming of organic matter as distinct from humic matter resulted in only a small increase in the available compounds, but that steaming of humic matter resulted in a large increase.

Analysis of the aqueous soil extract before and after steaming was made by a number of these early workers. Phosphate and nitrogen were found to be greatly increased, although some workers reported an increase in one form of nitrogen and a decrease in another.

Physical changes were also observed in the steamed soils. Kopeloff and Coleman (15) report that some early investigators found an increase in the absorptive capacity of steamed air dry soils and a decrease in steamed moist soils. They also report that steam heating for two hours at pressures from 1.5 to 2.5 atmospheres was found by Czernak to reduce the

soil surface and consequently the hygroscopicity by coagulating the soil colloids.

In the majority of cases, these results were obtained from comparatively severe treatments, with pressures ranging from 1 to 5 atmospheres, and from periods of 3 to 12 hours. It appears that the early investigators were interested only in establishing the fact that heat did induce changes in the soil, and in defining the nature of the changes. Little work was done with respect to the rate or degree of change over a range of temperatures.

With respect to the degree of change at various temperatures Kelley and McGeorge (12) reported increasing solubility of a number of minerals with increasing temperatures. They applied dry heat to different types of Hawaiian soils. Drying the soils at 100°C resulted in a small increase in the water soluble calcium, magnesium, manganese, phosphate, sulfate, and bicarbonate. The changes in potash, silica, and alumina were found to vary, with an increase in some soils and a decrease in others. In the majority of the soils tested, iron was found to decrease in solubility although in some a slight increase was recorded. At 150°C these chemical changes were somewhat intensified and nitrates began to decompose. At 200°C this decomposition of the nitrates was more extensive and at 250°C practically total destruction took place. The solubility of phosphorus appeared to reach a maximum at 200°C and at this temperature ammonia was produced in rather large amounts.

Kelley and Thompson (13) reported that steam heat applied to soils at 2 atmospheres pressure produced a similar chemical change to that produced by dry heat at 150°C . At 200°C all of the lateritic soils used in the investigation gave increases in ammonia. A few soils were found to have a decreased content of amide nitrogen while others were found to have an increased content after heating. Considerable losses in the amounts of non-basic nitrogen were found, but no changes in the amounts of basic nitrogen were observed.

Schreiner and Lathrop (27) have obtained some interesting results from their investigation of the chemistry of steam-heated soils. They steamed ten pounds of two silt loams at 30 pounds pressure (135°C) for 3 hours. On extracting the soil with a 2 per cent NaOH solution, they found a large increase in soluble solids. Analysis of the extract revealed an increase in the ammonia and amino acid content and, in addition, the presence of new organic compounds such as arginine, cytosine, guanine, xanthine, and hypoxanthine which were absent before heating. They concluded that these compounds resulted from the heat destruction of nucleic acid and the protein material of the soil. Dihydroxystearic acid was found to be greatly increased in one of the loams in which it was present originally, and a smaller amount was found in the other loam in which it was not present originally. This compound is known to be toxic to plants.

On analysing aqueous extracts from dry heated soils, Pickering (20, 21, 22) found that the soluble mineral and organic matter increased with temperatures ranging from 60°C to 250°C . The soluble nitrogen increased sharply after 100°C . He also noted that an increased moisture content of the soils had a tendency to intensify the change.

Russell and Hutchinson (26) observed that steaming a soil at atmospheric pressure (98°C) resulted in an increase in the unstable nitrogen compounds and therefore concluded that something more than a simple liberation of ammonia was involved. The increase in ammonia obtained was about five parts per million of soil. A smaller increase in nitrate was recorded.

Roch (14) used the depression of the Freezing Point as a means of measuring the amount of soluble matter in aqueous soil extracts. He observed that in both commercial and laboratory methods of steaming soils the heavier soils were more influenced by the treatments than the lighter soils.

Potter and Snyder (23) applied dry heat and steam heat under pressure to three different silt loams and a peat. Their results are consistent with those of other workers in that with the application of dry heat the ammonia and amino acid contents were increased, and in that the nitrates were increased slightly at 100°C but lost to the soils at 200°C . This also held true for the peat except that the relative increases were smaller, probably owing to the loss of ammonia to the atmosphere.

It is difficult to draw any definite conclusions from the results of Potter and Snyder as to the effect of increasing temperature on the steamed samples, as the results are confused with time. They used a 9 hour treatment at 10 pounds pressure, and a 3 hour treatment at 15 pounds pressure. Increases in all forms of nitrogen were, however, recorded, the larger increase being observed with the 10 pounds - 9 hour treatment. In contradiction, they found an increase in nitrate in the steamed soils instead of a decrease as reported by other workers.

Gustafson (7) dried soil in an electric oven at 105°C for 8 hours and found that the quantity of water soluble material removed by a one-to-five water extraction was increased to a marked degree. He found a sharp decrease in the amount of nitrate in the soil after heating. Evaporating a solution of potassium nitrate to dryness at 105°C also produced a distinct loss in nitrate.

In a study of the influence of heated soils on germination and plant growth, Johnson(9) observed that the toxic action of heated soils increased with temperature until a maximum was reached at 250°C , after which further temperature rise resulted in its quick disappearance. The concentration of the soil solution and the ammonia content of the soil was observed to follow the same pattern, which led him to conclude that they were roughly correlated with the degree of toxicity of the soils to seed germination and early plant growth. A review of the literature and further investigation led Johnson

to conclude that the ammonia was held in the soil as ammonium carbonate.

Heat induced physical changes in a wide range of soil types were studied by Bouyoucos (3). The soils were heated in an electric furnace at 100° , 170° , 230° , 485° , and 800°C for a period of 7 hours. After cooling, they were examined with regard to heat of wetting, water holding capacity, and plasticity. It was found that the three properties began to be affected at 230°C . At that temperature the changes commenced to be perceptible. At 485°C all three properties were greatly reduced and at 800°C they were destroyed.

Puri and Ashgar's (24) experimental results agreed with those of Bouyoucos in that complete destruction of the colloidal properties was attained at 800°C . After reaching 400°C they observed that the base exchange capacity declined rapidly with further temperature rise. At this temperature the humus was completely burned, and after 500°C the soils lost very little weight on further heating. A progressive decrease in hygroscopicity with increasing temperature was noted after 230°C . The failure of alkali saturated colloids to release alkali while being subjected to heat, led them to conclude that heating soils at high temperatures caused a fusion of the colloidal particles with a resultant loss of colloidal properties.

The results of Sreenivasan and Aurangabadkar (30) followed a similar pattern. They studied the physical changes

induced by heating a soil at 100° and 150°C , and by "fire-heating". A small decrease in the colloidal properties in the heated soil, and a large decrease in the "fire-heated" soil were reported. On investigating the chemical changes, the authors found little change in the carbon content of the soil up to 150°C , but "fire-heating" reduced the carbon by half. They recorded no change in the available potassium, phosphorus, or total nitrogen. Sreenivasan and Aurangabadkar suggested that the changes attendant on "fire-heating" the black clay of the Malwa region in India and the changes attendant on the application of humic manures were comparable.

Malowany (18) in a study of the changes caused by steaming four Alberta soils, reported little change in the physical properties. He did, however, obtain a significant decrease in the capillary rise of the four soils, and it is interesting to note that the decrease was greatest in the high organic soils and least in the low organic soils. Of the chemical changes studied, Malowany found no significant change in the pH, but an increase in the water soluble phosphorus and sulfate was observed. These results were obtained by autoclaving the soils in one gallon crocks at 15 to 17 pounds pressure for 45 minutes on the first day, and for 30 minutes on the second. This treatment, in comparison to that of other workers, was very light.

It would appear from a review of these investigations that the effect of the application of moist or dry heat on the physical condition of the soil was mainly that of fusing the

colloidal particles, thus decreasing the colloidal properties of the soil. The corresponding effect on the chemical constitution of the soil has been shown to be more complex. A number of workers have demonstrated an accumulation of ammonia and amino nitrogen and a loss in nitrate nitrogen on heating. Some investigators have reported the breakdown of organic and humic materials with the formation of new organic compounds. An increase in the solubility of a number of minerals has been observed while other minerals have been shown to decrease in solubility.

The results of the reported investigations suggest that heat induced changes begin to occur at temperatures as low as 60°C with a gradual increase in the degree of change until 200°C is reached, when chemical changes become intensive and the physical properties of the soil begin to be measurably affected. Heavier soils and those containing large percentages of organic and humic matter appear to be the most affected.

The practical problem of sterilizing soil by the application of heat is to reach all the particles of soil with such temperatures and exposures as will result in the death of the soil organisms. This necessitates periods of treatments far beyond those required for the actual destruction of the organisms.

Johnson (10) reasoned that the efficient steaming of a soil would depend upon the rate of direct penetration of the steam into the soil, and the subsequent conduction of the necessary heat to every particle of soil and living matter under

treatment. The period of application required would therefore be determined by the volume, the physical condition, and the moisture content of the soil sample. He also stressed the importance of the removal of air from the containing vessel and from the soil itself, as it would reduce the rate of steam penetration and the effective temperature.

Johnson (11) and Senner (28) regarded a high moisture content of the soil as detrimental to efficient sterilization. They called attention to the fact that five times as much steam is required to raise the temperature of a pound of water a given number of degrees as is required for the same weight of soil. In addition, they pointed out that steam cannot penetrate readily through wet soil, and the distribution of heat becomes dependent upon conduction, with resulting lowered efficiency. Johnson suggested that the ideal soil sterilizer should permit loose soil to pass through steam instead of steam being forced into the soil, thus aiding uniform penetration by the steam and reducing the necessary time of exposure.

Baver (2) reviewed the work of a number of investigators regarding factors affecting heat conductance in soils. Porosity of the soils was observed to have an inverse effect on the rate of conduction. An increase in pore space decreased the number of contacts between the soil particles and increased the air content with a resultant lowering of conductivity. It was observed that the heat conductivity of the different soils followed the order of sand, loam, clay, and peat, and that the thermal conductivity of the soil constituents, with the

possible exception of organic matter, varied little from one soil to another.

The presence of moisture in a soil was found by Patten (19) to result in an increased heat conductivity. Patten found that the conductivity increased sharply up to a certain moisture content, and then gradually reached a maximum at saturation, after which it tended to approach the conductivity of water. The point of inflection was found to vary with each soil.

OUTLINE OF THE INVESTIGATION

Three loam soils representing the Brown, the Black, and the Gray Wooded soil zones of Alberta were used in this study, in addition to a peat obtained from west of Edmonton. Samples of 50 gm., 100 gm., 400 gm., and 1000 gm. of soil on a water-free basis were respectively treated in 250 ml., 500 ml., 1000 ml., and 2000 ml. Erlenmeyers. The peat was also weighed on a water-free basis and 50 gm., 100 gm., and 250 gm. samples were treated in 500 ml., 1000 ml., and 2000 ml. Erlenmeyers.

The treatments consisted of autoclaving the samples daily at 12 or 15 pounds pressure. These consecutive daily treatments were given to a maximum of four days in the majority of the trials. Three different moisture levels were used; namely, air dry, 50 per cent and 100 per cent of the total water holding capacity. All samples except those treated in an air dry condition were weighed and brought to optimum moisture (50 per cent of water holding capacity) seven days prior to sterilization and incubated at room temperature. Such a procedure, it was reasoned, would bring the soil microflora to an active state and promote germination of the spores and cysts. The samples were checked as to their water content immediately before autoclaving, and those to be steamed at 100 per cent of water holding capacity were brought to this condition.

Using aseptic technique, the air dry samples were moistened with sterile water when sterilization treatments were completed. All samples were then incubated for twelve days at

room temperature before plating. The objection to plating immediately after steam treatment was that where only a few organisms survived, their detection in a large sample would be difficult. In addition, where sterilization had been very nearly achieved, the number of colonies developing on the plates would probably otherwise not have been much different from the number of colonies that could have been accredited to contamination from outside sources. In order to reduce the possibility of external contamination, transfers were made directly to plates from the flasks of soil to which sterile water had been added aseptically. All plates were incubated at 27° - 28°C for five days.

Plating was carried out in Petri dishes for all preliminary trials and confirming checks. To verify the sterility of the soil with respect to anaerobic organisms the spray anaerobic culture plate technique (29) was used in those treatments found to give sterility by the Petri plate method. Fred and Waksman's (6) sodium caseinate agar and beef-extract, peptone, glucose agar were used. All tests were made with duplicate flasks and verified three times, except in doubtful cases, where additional runs were made.

Of the chemical changes induced in the soil by the application of heat, that of base-exchangeable ammonia appeared to be the most affected. The change in ammonia content was thus used as an index of the degree of chemical change in the soil in order to select more accurately the sterilization treatments causing the least chemical change.

MATERIALS AND METHODS

Three loam soils representing the major color zones of the province of Alberta were selected for this investigation. The soils were collected during the summer of 1944 and replenished from the same locality during the summer of 1945. From Vauxhall, in the brown soil zone, a virgin loam was obtained. A loam from the black soil zone was taken from a cultivated field south of Edmonton. A third loam was obtained in the gray wooded soil zone from virgin soil near the Breton Plots. During the summer of 1945, a large sample of dark brown peat was obtained east of Winterburn.

After collection, the samples were air dried, and any large lumps broken by hand. Extraneous materials were removed from the soils by putting them through an eight mesh sieve. The peat, however, being somewhat coarse was not sieved, but was subjected to a moderate grinding in an iron soil grinder. The resultant samples were stored in large galvanized cans from which they were taken as required.

In order to correctly classify the soils as to texture, a mechanical analysis was conducted by the hydrometer method as outlined by Bouyoucos (4) and the results are described in Figure 1. It will be noted that the Edmonton black soil, a silty clay loam is the heaviest of the three soils. The Vauxhall brown silt loam, although quite similar in texture to the Breton gray soil, contains less sand and more silt.

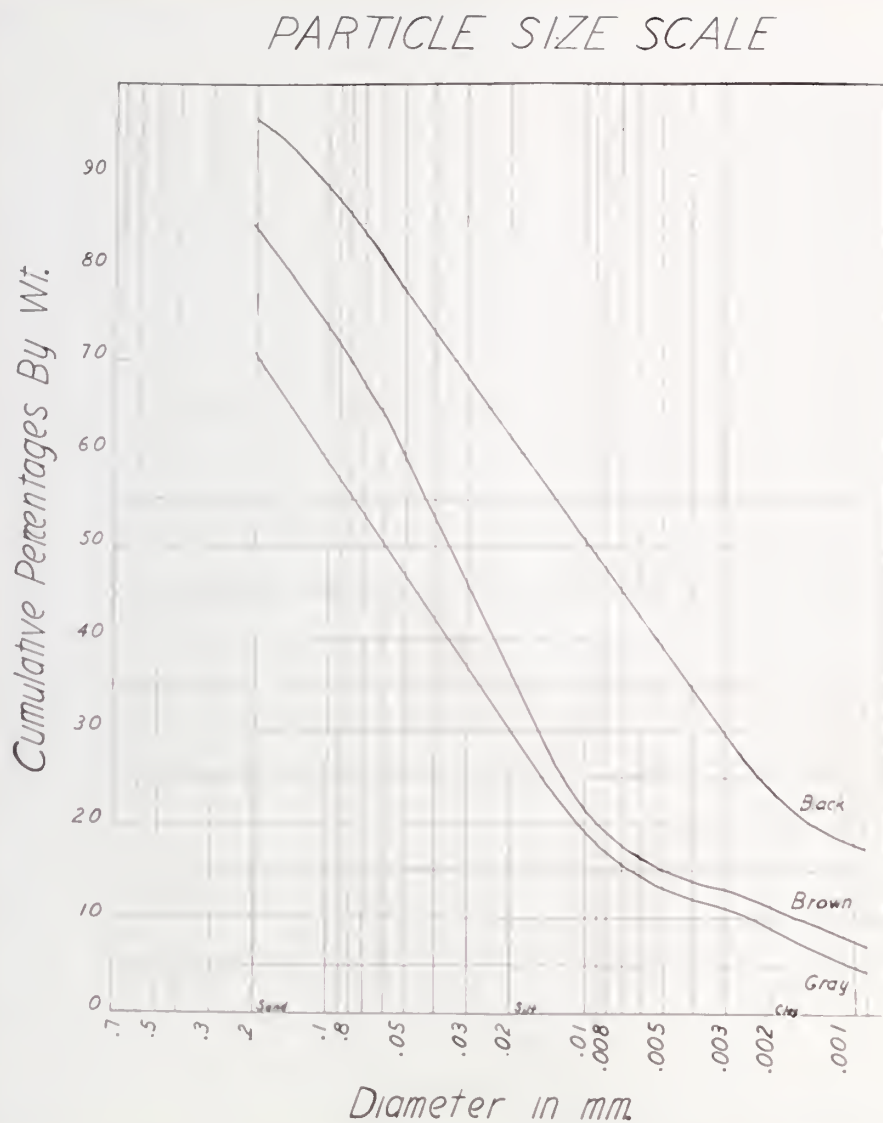


Figure 1. Mechanical Analysis of Edmonton Black, Vauxhall Brown, and Breton Gray-Wooded Soils.

In Table I the chemical composition of the three loam soils and the peat are compared (water-free basis). The soils are listed in order of increasing organic matter and this order is maintained throughout this report. The organic carbon was determined by the dichromate wet combustion method as outlined by Allison (1). Nitrogen was determined by the Kjeldhal method. Phosphorus was precipitated as $(\text{NH}_4)_3\text{PO}_4 \cdot 12\text{MoO}_3$ and determined volumetrically with standard NaOH and HNO_3 . Calcium was precipitated as CaC_2O_4 and titrated with standard KMnO_4 . Magnesium was precipitated as MgNH_4PO_4 and determined volumetrically with standard H_2SO_4 and NaOH. The pH values were determined by the quinhydrone electrode method.

Table I

Chemical Composition of Soils
(per cent, water-free basis)

Soil	Organic Carbon	Organic Matter*	N	P	Ca	Mg	pH
Breton gray loam	1.7	2.8	.16	.05	.86	.40	6.2
Vauxhall brown silt loam	2.0	3.5	.20	.07	.94	.52	7.0
Edmonton black silty clay loam	3.5	6.2	.50	.12	1.00	.58	7.2
Winterburn peat	53.7	92.6	2.42	.03	2.02	.48	6.6

* Organic Carbon x factor 1.724

A Bramhall Deane autoclave was used for treating the samples, and operated according to techniques described by Underwood (3). The Erlenmeyers containing the samples to be steamed were placed in the autoclave on their sides to permit a more efficient removal of air from the flask and the soil.

The autoclave had two disadvantages in that it was equipped with a pressure gauge that took the pressure from the surrounding steam jacket and not from the sterilizing chamber, and in that it was not equipped with a shut-off valve to control the admittance of steam into the sterilizing chamber. The position of the pressure gauge may have resulted in a small error in so far as the pressure in the autoclave jacket and the pressure in the sterilizing chamber might not have been equal. It is, however, doubtful if this difference in pressure would be maintained for any length of time.

The absence of a shut-off valve between the jacket and the chamber prevented accurate control of the period of time the soils were actually subjected to steaming. Although the precaution was taken to see that the water in the boiler was steaming before the soils were placed in the sterilizing chamber, a considerable warming-up period was still necessary to raise the pressure and temperature to that required. This warming-up period varied from 4 to 6 minutes for achieving 12 pounds pressure, and from 7 to 10 minutes for achieving 15 pounds pressure. The period of time that the soils were

subjected to steaming was taken from the moment the required pressure was reached. Care was taken to keep the pressure constant.

A mercury thermometer was attached to the discharge line to record the temperature of the coolest part of the autoclave. It was observed on all occasions that the temperature in the discharge line at the moment the desired pressure was reached lagged behind the theoretical temperature by 6° to 7°C . This lag, however, soon decreased to within 4° , where it remained constant. It was observed that an increase in the size of load placed in the autoclave increased the time required to obtain the minimum lag, and also increased the length of the warming-up period. The size of load was therefore kept as small as possible.

Because of the fact that no single plating medium is known that will enable all of the different soil bacteria to grow, two different media were used for this investigation. Fred and Waksman's (6) sodium caseinate agar, and beef-extract, peptone, glucose agar were used. Both agars were used for plating the samples in the aerobic Petri and anaerobic Spray culture dishes. The plates were counted after a five day incubation period at $27^{\circ} - 28^{\circ}\text{C}$.

Ammonia was determined by the McLean and Robinson (17) method. Fifty grams of soil on a water-free basis were leached with 500 millilitres of normal sodium chloride solution, and the resulting extract distilled with magnesium oxide. The ammonia was collected in standard acid and the excess

titrated with a standard base, using methyl red and methylene blue. Distillation was carried on until approximately 250 millilitres of distillate were collected. The 50 gram samples were analysed immediately on cooling after the same treatment. The hundred gram samples when cool were brought to a desired moisture content and amounts equal to 50 grams on a water-free basis were weighed and analysed. In the case of the peat, 750 millilitres of normal sodium chloride were used.

Nitrate determinations were carried out colorimetrically by the phenol-diazotonic method as modified by Harper (8). One-to-five water extractions were used.

The ammonia and nitrate determinations were made on quadruplicate samples. The samples were incubated four days before being sterilized. Duplicate controls were run and subjected to the same treatment as the steamed soils except that of steaming.

RESULTS

Requirements for the Steam Sterilization of Soils

The number of consecutive daily treatments and the length of the treatments required to sterilize the three soils and the peat at three different moisture contents are given in Tables II to X. Each table contains the data of one size of sample sterilized at one stated pressure except in the case of the 250 gram sample of peat, where the requirements for obtaining sterility at the two pressures are given in the one table.

A graphical illustration of the relationship between the different soils and the peat, the size of sample, the moisture content, and the time required to sterilize at 12 pounds pressure is made in Figure 2. Figure 3 illustrates the relationships of the same factors at 15 pounds pressure. Both graphs were drawn to the same scale to facilitate a comparison of the results obtained with 12 pounds and 15 pounds pressure.

Table II.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(50 gm. of Soil at 12 lb. Pressure (118°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes										
		15	20	30	45	60	75	90	105	120	150	180
Gray loam	4**	N*	3	2	1	1	-	-	-	-	-	-
	50	N	3	2	1	1	-	-	-	-	-	-
	100	N	3	2	1	1	-	-	-	-	-	-
Brown silt loam	3**	N	3	2	1	1	-	-	-	-	-	-
	50	N	3	2	1	1	-	-	-	-	-	-
	100	N	3	2	1	1	-	-	-	-	-	-
Black silty clay loam	9**	N	3	2	1	1	-	-	-	-	-	-
	50	N	3	2	1	1	-	-	-	-	-	-
	100	N	3	2	1	1	-	-	-	-	-	-
Peat	6**	-	-	-	N	N	N	N	3	2	2	1
	50	-	-	-	N	3	3	2	2	2	1	1
	100	-	-	-	N	3	3	2	2	2	1	1

* N - insufficient to sterilize.

** Air dry.

Table III.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(50 gm. of Soil at 15 lb. Pressure (121°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes									
		10	15	20	30	45	60	75	90	105	120
Gray loam	4**	3	1	1	1	-	-	-	-	-	-
	50	1	1	1	1	-	-	-	-	-	-
	100	1	1	1	1	-	-	-	-	-	-
Brown silt loam	3**	N*	2	1	1	-	-	-	-	-	-
	50	N	2	1	1	-	-	-	-	-	-
	100	N	2	1	1	-	-	-	-	-	-
Black silty clay loam	9**	N	2	1	1	-	-	-	-	-	-
	50	N	2	1	1	-	-	-	-	-	-
	100	N	2	1	1	-	-	-	-	-	-
Peat	6**	-	-	N	N	3	2	2	2	2	1
	50	-	-	N	3	2	2	1	1	1	1
	100	-	-	N	3	2	1	1	1	1	1

* N - insufficient to sterilize.

** Air dry.

Table IV.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(100 gm. of Soil at 12 lb. Pressure (118°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes												
		20	30	45	60	75	90	105	120	135	150	165	180	240
Gray loam	4**	N*	3	2	1	1	-	-	-	-	-	-	-	-
	50	N	3	2	1	1	-	-	-	-	-	-	-	-
	100	N	2	1	1	1	-	-	-	-	-	-	-	-
Brown silt loam	3**	N	N	3	2	1	-	-	-	-	-	-	-	-
	50	N	N	3	2	1	-	-	-	-	-	-	-	-
	100	N	N	3	2	1	-	-	-	-	-	-	-	-
Black silty clay loam	9**	N	N	4	3	2	1	-	-	-	-	-	-	-
	50	N	N	4	3	2	1	-	-	-	-	-	-	-
	100	N	N	4	3	2	1	-	-	-	-	-	-	-
Peat	6**	-	-	N	N	N	N	3	3	2	2	2	2	1
	50	-	-	N	N	3	2	2	2	2	1	1	1	1
	100	-	-	N	3	2	2	2	2	2	1	1	1	1

* N - insufficient to sterilize.

** Air dry.

Table V.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(100 gm. of Soil at 15 lb. Pressure (121°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes.									
		10	15	20	30	45	60	75	90	105	120
Gray loam	4**	3	2	2	1	1	-	-	-	-	-
	50	1	1	1	1	1	-	-	-	-	-
	100	1	1	1	1	1	-	-	-	-	-
Brown silt loam	3**	**	3	2	2	1	-	-	-	-	-
	50	N	3	2	1	1	-	-	-	-	-
	100	N	3	2	1	1	-	-	-	-	-
Black silty clay loam	9**	N	N	3	2	1	-	-	-	-	-
	50	N	N	3	2	1	-	-	-	-	-
	100	N	N	3	2	1	-	-	-	-	-
Peat	6**	-	N	N	N	N	2	2	2	1	1
	50	-	N	3	2	1	1	1	1	1	1
	100	-	N	3	2	1	1	1	1	1	1

* N - insufficient to sterilize.

** Air dry.

Table VI.

Number of Consecutive Daily Treatments Required to
Sterilize Soils at Different Moisture Contents
(250 gm. of Peat)

Autoclave Pressure	Moisture, % Water Capacity	Length of Treatment in Minutes									
		45	60	90	120	150	180	210	240	270	300
12 lb. (118°C.)	6**	-	-	-	N*	N	N	3	2	2	1
	50	-	-	-	N	3	2	2	1	1	1
	100	-	-	-	N	3	2	2	1	1	1
15 lb. (121°C.)	6**	N	N	N	4	2	2	1	-	-	-
	50	N	4	2	2	1	1	1	-	-	-
	100	N	4	2	1	1	1	1	-	-	-

* N - insufficient to sterilize.

** Air dry.

Table VII.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(400 gm. of Soil at 12 lb. Pressure (118°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes						
		30	45	60	75	90	120	150
Gray loam	4**	N*	4	3	2	2	1	-
	50	N	4	3	1	1	1	-
	100	N	4	3	1	1	1	-
Brown silt loam	3**	N	N	4	3	2	1	-
	50	N	N	3	2	1	1	-
	100	N	N	3	2	1	1	-
Black silty clay loam	9**	N	N	N	3	2	2	1
	50	N	N	4	3	2	1	1
	100	N	N	4	3	2	1	1

* N - insufficient to sterilize.

** Air dry.

Table VIII.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(400 gm. of Soil at 15 lb. Pressure (121°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes						
		20	30	45	60	75	90	120
Gray loam	4**	N*	3	2	1	-	-	-
	50	N	1	1	1	-	-	-
	100	N	1	1	1	-	-	-
Brown silt loam	3**	N	N	3	2	1	-	-
	50	N	N	2	1	1	-	-
	100	N	N	2	1	1	-	-
Black silty clay loam	9**	N	N	N	N	3	2	1
	50	N	N	N	4	3	1	1
	100	N	N	N	4	3	1	1

* N - insufficient to sterilize.

** Air dry.

Table IX.

Number of Daily Consecutive Treatments Required to
Sterilize Soil at Different Moisture Contents
(1000 gm. of Soil at 12 lb. Pressure (118°C.))

Soil	Moisture, % Water Capacity	Length of Treatment in Minutes								
		45	60	90	120	150	180	210	240	270
Gray loam	4**	N	N	N	3	2	2	1	1	-
	50	N	4	3	2	2	1	1	1	-
	100	N	4	2	2	1	1	1	1	-
Brown silt loam	3**	N	N	N	3	2	2	2	1	-
	50	N	N	4	2	2	2	1	1	-
	100	N	N	4	2	2	2	1	1	-
Black silty clay loam	6**	N	N	N	4	3	3	2	2	1
	50	N	N	4	2	2	2	2	1	1
	100	N	N	4	2	2	2	2	1	1

* N - insufficient to sterilize.

** Air dry.

Table X.

Number of Consecutive Daily Treatments Required to
Sterilize Soil at Different Moisture Contents
(1000 gr. of Soil at 15 lb. Pressure (121°C.))

Soil	Moisture, % water Capacity	Length of Treatment in Minutes							
		45	60	90	120	150	180	210	240
Gray loam	4**	N*	3	2	2	1	-	-	-
	50	N	1	1	1	1	-	-	-
	100	N	1	1	1	1	-	-	-
Brown silt loam	3**	N	N	N	3	2	1	-	-
	50	N	3	1	1	1	1	-	-
	100	N	3	1	1	1	1	-	-
Black silty clay loam	9**	N	N	N	N	4	3	2	1
	50	N	3	2	1	1	1	1	1
	100	N	3	1	1	1	1	1	1

* N - insufficient to sterilize.

** Air dry.

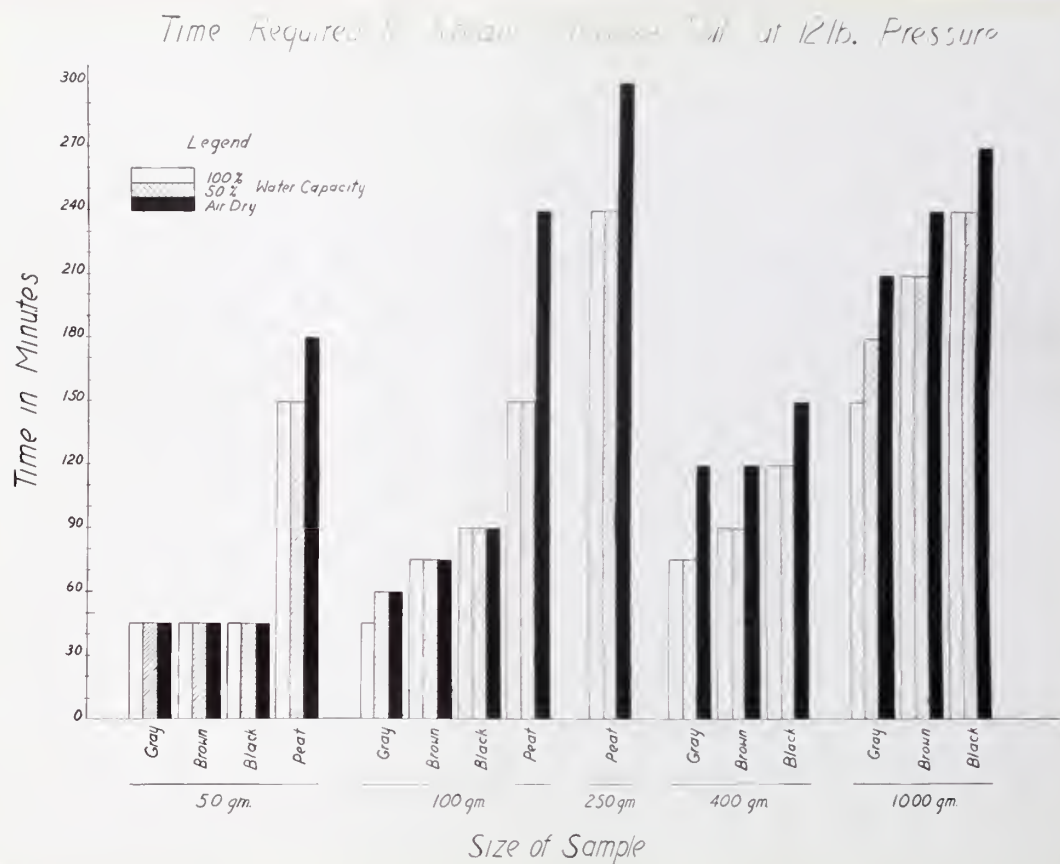


Figure 2. The Effect of Size of Sample, Kind of Soil, and Moisture Content on the Time Required to Steam Sterilize at 12 lb. Pressure.

Time Required to Steam Sterilize Soils at 15 lb. Pressure

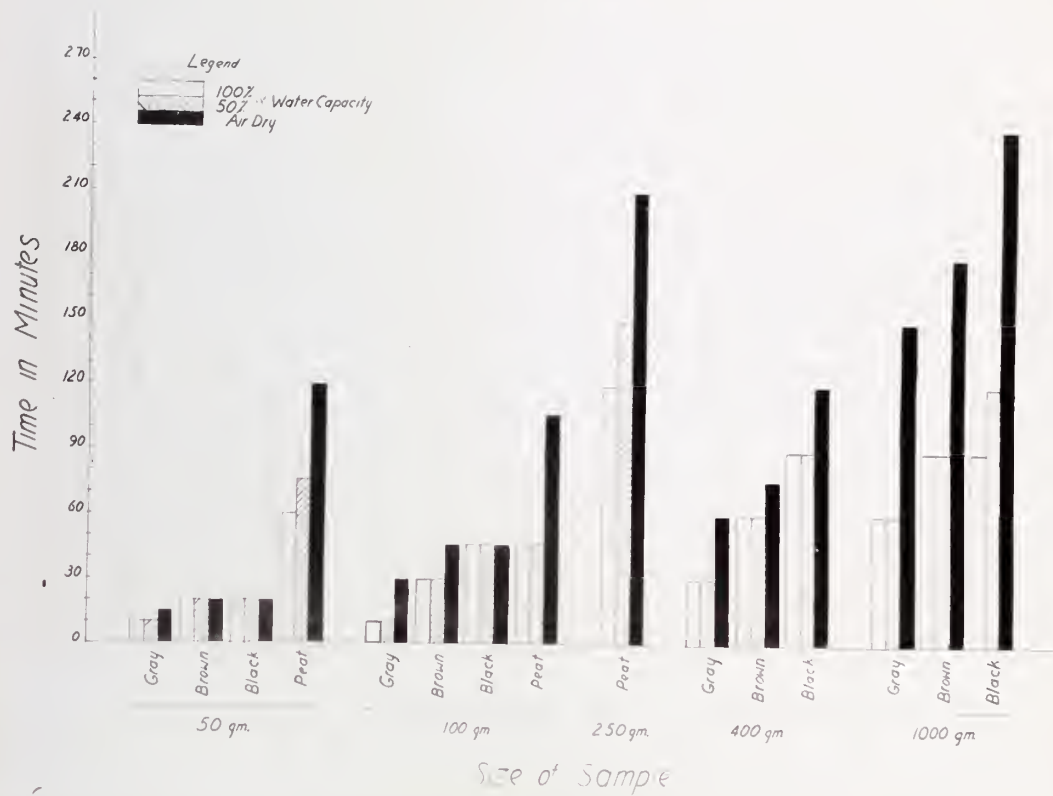


Figure 3. The Effect of Size of Sample, Kind of Soil, and Moisture Content on the Time Required to Steam Sterilize at 15 lb. Pressure.

Changes in the Base-Exchangeable Ammonia
and Water Soluble Nitrate Content of Soils
Induced by Steaming Under Pressure

The average ammonia content of the three soils and the peat in non-sterilized and in sterilized samples are given in Tables XI and XII. The figures represent the average amounts of ammonia found in samples subjected to the minimum treatments required to achieve sterility. The amounts of ammonia in the non-sterilized samples were determined on duplicate controls which received the same treatment as the sterilized samples except that of autoclaving.

In Tables XIII to XVI inclusive, are given the average increases in ammonia resulting from subjecting quadruplicate samples of 50 and 100 grams to the predetermined minimum sterilization treatments.

To facilitate a comparison of the effect of the moisture content and the number of consecutive daily treatments on the production of ammonia on sterilizing, the increases found for the three soils were averaged for each moisture content and each number of consecutive treatments. The resulting figures are given in Table XVII.

A number of determinations were made to determine the changes in the water soluble nitrate content of the 50 gram samples of soil subjected to steam sterilization. The amounts of nitrate found in the non-sterilized controls and in the sterilized samples are reported in Table XVIII.

Table XI.

Base-Exchangeable Ammonia in Soils Sterilized
at 12 lb. Pressure and in Non-Sterilized Controls

Wt. of Sample in gm.	Moisture, % water capacity	Ammonia (NH ₃) - p.p.m. of Soil							
		Gray loam		Brown silt loam		Black silty clay loam		Peat	
		Con- trol	Steri- lized	Con- trol	Steri- lized	Con- trol	Steri- lized	Con- trol	Steri- lized
50	Air Dry	21.9	25.1	18.8	26.4	12.0	19.6	91.2	151.6
	50	33.1	36.1	16.8	29.3	4.3	13.1	97.3	202.2
	100	32.8	36.8	16.4	29.9	4.1	20.0	109.4	204.4
100	Air Dry	22.0	23.1	10.7	11.8	9.8	22.5	-	-
	50	22.3	27.4	8.6	15.1	7.6	27.6	-	-
	100	23.1	28.6	9.1	15.0	9.2	28.1	-	-

Table XII.

Base-Exchangeable Ammonia in Soils Sterilized
at 15 lb. Pressure and in Non-Sterilized Controls

Wt. of Sample in gm.	Moisture, % water capacity	Ammonia (NH ₃) - p.p.m. of Soil							
		Gray loam		Brown silt loam		Black silty clay loam		Peat	
		Con- trol	Steri- lized	Con- trol	Steri- lized	Con- trol	Steri- lized	Con- trol	Steri- lized
50	Air Dry	6.0	7.1	6.0	6.8	5.2	8.0	90.5	108.1
	50	5.9	9.5	3.5	6.9	5.9	11.1	96.0	154.7
	100	5.0	10.2	3.2	7.4	5.5	11.2	106.0	131.2
100	Air Dry	13.2	15.1	5.3	10.1	6.2	13.5	-	-
	50	9.5	13.9	7.1	12.4	6.4	19.3	-	-
	100	9.6	16.6	7.1	13.5	5.4	19.6	-	-

Table XIII.

Increases in Soil Ammonia Produced by Steam Sterilization
(50 gm. of Soil at 12 lb. Pressure (118°C.))

No. of consecu- tive daily treatments	Length of treat- ment in minutes	Moisture, % water capacity	Ammonia(NH ₃) Incr. - p.p.m. Soil			
			Gray loam	Brown silt loam	Black silty clay loam	Peat
3	20	Air Dry	2.5	6.8	8.7	-
3	20	50	2.3	12.3	16.4	-
3	20	100	3.2	13.1	21.5	-
2	30	Air Dry	2.9	7.4	7.5	-
2	30	50	2.2	12.6	14.9	-
2	30	100	3.3	13.2	13.3	-
1	45	Air Dry	1.1	7.4	7.3	-
1	45	50	5.0	12.8	11.8	-
1	45	100	4.8	11.8	13.0	-
1	60	Air Dry	6.2	9.5	7.6	-
1	60	50	5.6	13.5	12.4	-
1	60	100	8.1	14.2	15.9	-
3	105	Air Dry	-	-	-	73.3
3	60	50	-	-	-	160.2
3	60	100	-	-	-	137.9
2	150	Air Dry	-	-	-	49.2
2	90	50	-	-	-	115.4
2	90	100	-	-	-	81.0
1	180	Air Dry	-	-	-	40.9
1	150	50	-	-	-	43.0
1	150	100	-	-	-	34.9

Table XIV.

Increases in Soil Ammonia Produced by Steam Sterilization
(50 gm. of Soil at 15 lb. Pressure (121°C.))

No. of consecu- tive daily treatments	Length of treat- ment in minutes	Moisture, % water capacity	Ammonia(NH ₃)		Incr. - p.p.m. Soil	
			Gray loam	Brown silt loam	Black silty clay loam	Peat
3	10	Air Dry	12.6	-	-	-
1	10	50	4.4	-	-	-
1	10	100	4.8	-	-	-
2	15	Air Dry	2.9	2.0	6.4	-
2	15	50	-	1.7	12.7	-
2	15	100	-	2.8	12.9	-
1	20	Air Dry	1.7	4.8	4.8	-
1	20	50	3.6	4.7	9.8	-
1	20	100	8.3	4.8	9.9	-
3	45	Air Dry	-	-	-	21.0
2	60	Air Dry	-	-	-	19.0
2	45	50	-	-	-	42.0
2	45	100	-	-	-	38.6
1	150	Air Dry	-	-	-	21.6
1	60	50	-	-	-	108.0
1	60	100	-	-	-	98.1

Table XV.

Increases in Soil Ammonia Produced by Steam Sterilization
(100 gm. of Soil at 12 lb. Pressure (118°C.))

No. of consecu- tive daily treatments	Length of treat- ment in minutes	Moisture, % water capacity	Ammonia(NH ₃) Incr. - p.p.m. Soil		
			Gray loam	Brown silt loam	Black silty clay loam
3	30	Air Dry	3.7	-	-
3	30	50	6.1	-	-
2	45	Air Dry	6.3	-	-
2	45	50	9.4	-	-
2	30	100	6.5	-	-
1	60	Air Dry	2.6	-	-
1	60	50	5.0	-	-
1	45	100	3.0	-	-
3	45	Air Dry	-	1.3	-
3	45	50	-	4.7	-
3	45	100	-	4.9	-
2	60	Air Dry	-	1.3	-
2	60	50	-	8.8	-
2	60	100	-	9.1	-
1	75	Air Dry	-	1.0	-
1	75	50	-	3.7	-
1	75	100	-	4.1	-
3	60	Air Dry	-	-	17.3
3	60	50	-	-	24.8
3	60	100	-	-	25.0
2	75	Air Dry	-	-	10.3
2	75	50	-	-	20.1
2	75	100	-	-	19.8
1	90	Air Dry	-	-	10.8
1	90	50	-	-	15.0
1	90	100	-	-	11.9

Table XVI.

Increases in Soil Ammonia Produced by Steam Sterilization.
(100 gm. of Soil at 15 lb. Pressure (121°C.))

No. of consecu- tive daily treatments	Length of treat- ment in minutes	Moisture, % water capacity	Ammonia(NH ₃) Incr. - p.p.m. Soil		
			Gray loam	Brown silt loam	Black silty clay loam
3	10	Air Dry	3.7	-	-
1	10	50	8.7	-	-
1	10	100	5.6	-	-
2	15	Air Dry	1.0	-	-
1	15	50	4.1	-	-
1	15	100	3.4	-	-
3	20	Air Dry	-	5.8	9.2
3	20	50	-	-	16.5
3	20	100	-	-	17.5
2	20	50	-	5.9	-
2	20	100	-	6.4	-
2	30	Air Dry	-	5.8	6.0
2	30	50	-	-	8.6
2	30	100	-	-	10.9
1	30	Air Dry	2.2	-	-
1	30	50	2.4	4.1	-
1	30	100	3.7	5.7	-
1	45	Air Dry	-	2.3	6.2
1	45	50	-	-	14.0
1	45	100	-	-	14.8

Table XVII.

Effect of Moisture Content and Number of Consecutive Treatments on the Ammonia Produced by Steam Sterilization.

Moisture, % water capacity	No. of consecu- tive daily treatments	Average Ammonia (NH ₃) Increases - p.p.m. of Soil	
		12 lb. Pressure (118°C.)	15 lb. Pressure (121°C.)
Air Dry	1	5.0	3.7
	2	5.9	4.0
	3	6.7	7.8
50	1	8.8	7.3
	2	11.3	7.2
	3	11.1	16.5*
100	1	8.1	7.6
	2	11.0	8.2
	3	13.8	17.5*

* Edmonton black only (not an average of the three soils.)

Table XVIII.

Water Soluble Nitrate Nitrogen in 50 gm. of Sterilized and Non-Sterilized Soils

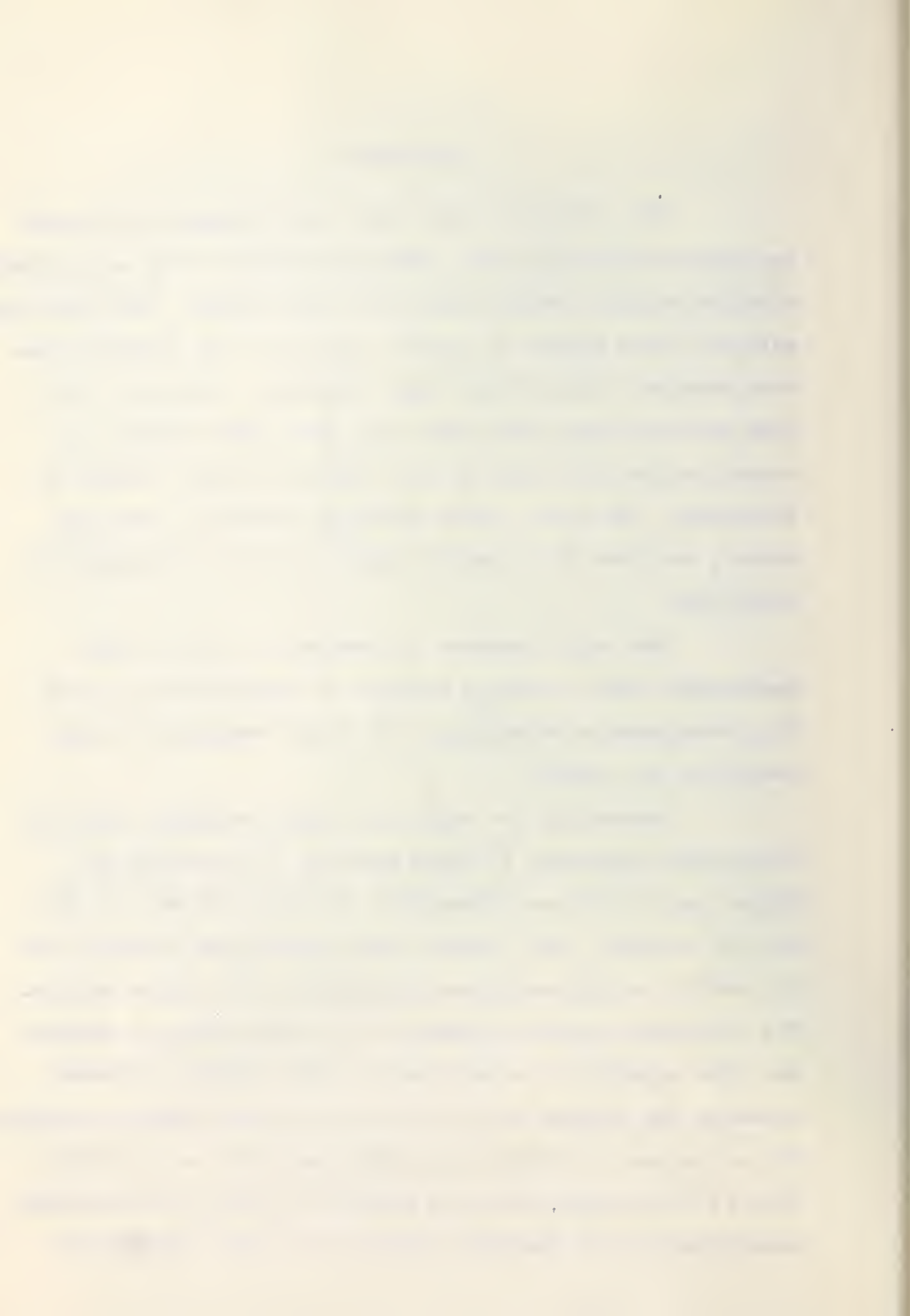
Pressure of Autoclave	Moisture, % water capacity	Nitrate Nitrogen - p.p.m. of Soil					
		Gray loam		Brown silt loam		Black silty clay loam	
		Con- trol	Steri- lized	Con- trol	Steri- lized	Con- trol	Steri- lized
12 lb. (118°C.)	Air Dry	3.0	3.1	22.4	23.1	32.0	34.4
	50	3.0	3.1	20.0	18.6	42.4	38.7
	100	3.2	2.7	23.2	18.9	42.3	38.6
15 lb. (121°C.)	Air Dry	3.4	3.6	21.3	20.6	32.0	31.8
	50	3.7	4.0	22.3	20.0	32.8	30.0
	100	4.0	3.2	22.0	18.2	35.0	34.3

DISCUSSION

The difficulty with which the different soils used in this investigation were sterilized appears to be correlated with the organic matter content and the texture. The gray loam with the least amount of organic matter and the coarsest texture required the shortest steam treatment. The brown silt loam and the black silty clay loam with larger amounts of organic matter and finer texture required longer periods of treatment. The peat, almost entirely composed of organic matter, required the severest steaming for the attainment of sterility.

The small increase in pressure of three pounds accompanied with a similar increase in temperature resulted in a considerable reduction in the time necessary to steam sterilize the soils.

Incubating the soils in a moist condition prior to autoclaving decreased the time required to sterilize the larger samples by comparison with the time required for the air dry samples. The shorter time required was probably due in part to the improved heat conduction of the moist samples. The reduction was most evident in the peat, where it reduced the time required by as much as 30 to 90 minutes. However, steaming the samples at 100 per cent of water holding capacity did not appear to improve the efficiency much over steaming them at 50 per cent, as in the majority of cases both moisture conditions had an identical effect on the time required to



achieve sterility.

The incubation period of one week is a comparatively long one and it is conceivable that a shorter period may be as efficient. It is not known what effect incubation alone has on the length of time required to sterilize the samples. Consequently the effect of moisture may be attributed in part to the lower heat resistance of an active microflora resulting from incubating the samples at optimum moisture. However, the observation that incubation of the 50 and 100 gram moist samples did not appear to reduce the time required as compared with the air dry samples, would seem to indicate that incubation was of minor importance and that steam penetration or heat conductance was of greater importance.

This leads to the question of the actual mechanism by which the heat necessary to kill the soil organisms was transferred through the soil. If the mechanism was largely that of steam penetration and subsequent conduction of the heat to the centre of each soil particle, then the efficient removal of air from the containing vessel and the soil would be of great importance, and the moisture content of the soil of minor importance. A high moisture content would probably be detrimental to the process.

An attempt was made to determine whether steam penetration and subsequent heat conduction or heat conduction alone was the important factor in steam sterilization of soils. One series of 2000 millilitre Erlenmeyers containing 1000 grams

of air dry or moist black soil was placed on their sides in the autoclave, while another series of flasks was placed upright in the autoclave. The flasks were treated for varying lengths of time differing by ten minutes. It was reasoned that the flasks lying on their sides would allow a more efficient removal of air with a resultant shorter period of treatment necessary to obtain sterility.

No differences were obtained with the moist samples. The air dry samples, however, were more efficiently sterilized in the flasks lying on their sides than in those placed upright in the autoclave. It would thus appear that steam penetration is an important factor in sterilizing air dry soils, but that conduction is of greater importance in sterilizing moist soils. The conductance of heat through a moist soil in actual practice thus appears to be a more effective process than steam penetration. If this is true, it should be possible to obtain the same results as found in this investigation by heating the moist soils in a dry atmosphere to the same temperature as that obtained in the autoclave.

The use of thermocouples and a potentiometer in measuring the temperatures of the soil during the process of sterilization would have permitted a more accurate comparison of the tests and an immediate analysis of the results. Their use would also have eliminated much of the empirical work required to establish the general limits for minimum sterilization. Unfortunately, the construction of the autoclave would

not allow their use without a major alteration.

The shorter length of time required to sterilize moist soil may be explained on the basis of a more heat susceptible microflora and a more efficient transfer of heat by conduction through the soil with the conversion of the soil moisture into steam and its subsequent destructive action on the microflora. As the soil moisture is already well distributed throughout the soil the difficult problem of forcing steam into the soil is eliminated. In addition, as the soil particles are moist, the efficient conduction of the heat to all parts of the soil material is assured.

Some support to this may be given by a curious observation. At the end of a treatment, water was observed to be floating on the surface of the larger samples steamed at 100 per cent of water holding capacity. The water disappeared instantaneously into the soil when the flasks were hit with a draft of air at room temperature. This phenomenon may be explained by reasoning that the moisture held in the interstices of the soil was converted to steam which forced unconverted moisture out of the soil to the cooler region of the steam-air mixture above the sample. A sudden condensation and release of pressure would account for the quick disappearance of the water into the soil.

The different sterilization requirements for the three soils can be more easily explained on the basis of conduction than of steam penetration. Baver (2) reported

that the conductivity of the different soil constituents were found to be, in decreasing order, sand, silt, clay, and organic matter. Thus on the basis of conduction, one would expect the gray soil to require the least treatment.

The degree of compaction also was reported by Bayer to have a direct effect on the heat conductivity of soils. The gray and brown soils were observed to settle on incubation to a similar degree of compaction. The black soil was characterized by a more porous condition and swelled considerably on wetting. This swelling was also characteristic of the peat. Thus the natural porosity of the different soils would indicate that the gray loam and the brown silt loam would be more easily sterilized, on the assumption that heat conductivity was the important factor in steam sterilization.

The different requirements of the three soils and the peat are somewhat more difficult to explain on the basis of steam penetration. One would expect steam to displace the air and penetrate more readily through a loose porous soil than a compact one. Yet the air dry samples with the loose crumb-like structure (except the peat) required more time than the amorphous appearing moist soils. This crumb-like structure was most evident in the black soil where the particles were of much larger size than in the gray or brown soils.

In all cases, as is shown in Tables XI and XII, steaming under pressure resulted in an increase in the ammonia content of the three soils and the peat. It is interesting

to note that incubating the unsterilized soils in a moist condition for four days resulted in a reduction in the amount of ammonia present. This may be owing to the conversion of the ammonia to nitrate or to fixed forms of nitrogen by the soil organisms. In two cases the peat drop loss produced more ammonia on incubation. The peat also produced more ammonia on incubation. This gray loam soil was observed to have an active fungus flora, and this may account for the production of ammonia (32). No extensive growths of fungi were observed in the peat samples, but the increase in ammonia on incubation is probably owing to an active soil-dwelling flora.

The comparatively large amounts of ammonia reported in Table XI as compared with those in Table XII may be explained by the fact that the ammonia determinations on the samples sterilized at 15 pounds pressure and the controls were made during the early summer of 1945, while the determinations on the samples sterilized at 10 pounds pressure and the controls were made in February, 1946.

In Tables XIII to XVI inclusive, the figures given represent the increase in ammonia content caused by steaming the soils and are listed for the various moisture treatments at 10 and 15 pounds pressure. Generally speaking, a higher moisture content seems to result in a larger amount of ammonia being produced on steaming. There are exceptions, however, the most notable being observed in the peat, where samples treated at 100 per cent of water holding capacity produced

much less ammonia than those treated at 50 per cent.

It is interesting to note that regardless of the additional time required, sterilizing the samples in an air dry condition resulted in the production of less ammonia than sterilizing the samples in a moist condition. This observation is more clearly brought out in Table XVII, where the increases for the three soils are averaged. It would appear from these averages that one minimum treatment results in a smaller increase than two or three consecutive treatments, despite the fact that the consecutive treatments are for shorter periods. Some support for this assumption may be obtained from McCulloch (16) who stated that short single periods of exposure to steam under pressure have been shown to cause less breaking down of certain complex carbohydrates in aqueous solution than steaming for intermittent periods sufficiently long to insure sterility.

The figures in Table XVII also indicate that less ammonia is released by sterilizing at the higher pressure of 18 pounds for a shorter period of time than at the lower pressure of 12 pounds for a longer period. Not only does this appear true for the one sterilization treatment, but also appears true for the two and three consecutive treatments. The two exceptions noted are not representative figures, as they were obtained with only one soil, the black silty clay loam, and are not averages of the increases for the three soils. Referring back to Tables XIII, XIV, XV, and XVI,

a number of exceptions to these conclusions can be noted.

The results reported for the water soluble nitrate content of the sterilized 50 gram samples showed much variability. Both increases and decreases were found to result from sterilizing at 12 pounds pressure. Sterilizing at 15 pounds pressure appeared to result in a more consistent loss in nitrate. The amount of loss, however, was quite variable.

It would thus appear that steaming soils under pressure causes a disappearance of nitrate. This is in agreement with the results of a number of workers, but is not in agreement with the findings of Potter and Snyder (23) who reported definite increases in nitrate on steaming under pressure.

Using the increase in the amount of base-exchangeable ammonia as an indication of the degree of chemical change induced by steaming under pressure, it would appear that the least chemical change would be obtained by autoclaving the samples in an air dry condition at 15 pounds pressure for the minimum length of time required to sterilize in one treatment. The minimum sterilization treatments for each soil and weight of sample resulting in the least increase in ammonia are given in Table XIX.

As is observed in Table XIX and in Figure 3, the 100 gram samples of peat appear to require less time to sterilize at 15 pounds pressure than the 50 gram samples. This is not only true for the air dry samples but also for those incubated

Table XIX

Minimum Sterilization Treatments Resulting
in the Least Increase in Ammonia

Length of Time Required to Sterilize Air Dry Samples
at 15 lb. Pressure (121°C)

Weight of Sample in grams	Time in Minutes			
	Gray loam	Brown silt loam	Black silty clay loam	Peat
50	15	20	20	120
100	30	45	45	105
250	-	-	-	210
400	60	75	120	-
1000	150	180	240	-

and sterilized in a moist condition. Sterilizing the 50 and 100 gram samples in a moist condition at 12 pounds pressure requires the same amount of time for each weight.

These abnormalities may be explained by the observation that the 50 gram samples filled the 500 millilitre Erlenmeyers to a greater depth than the 100 gram samples filled the 1000 millilitre Erlenmeyers. Despite the fact that the results were verified, the stated times required to sterilize 100 grams of air dry and moist peat should be accepted with some reservation.

The use of steam sterilized soil as a natural medium for the study of soil micro-organisms appears to be quite feasible. The small changes in the ammonia and nitrate nitrogen contents of the soils caused by the minimum sterilization treatments are not of greater magnitude than the normal variations found to occur in soil under natural conditions. As the application of heat appears to affect other chemical changes to a lesser degree than that of ammonia production (7, 15), it would seem that steam sterilizing soils without prohibitive chemical changes is achievable. Before a definite conclusion can be made, however, a more extensive survey of the chemical and physical changes induced by the various minimum steam sterilization treatments would be necessary.

SUMMARY

1. The requirements for steam sterilization of three Alberta loam soils and a peat were investigated and the results reported.

2. The amount of organic matter and the texture of the three loam soils appeared to have a distinct effect on the length of treatment required to sterilize the larger samples.

3. Incubating and steaming the soils in a moist condition was found to reduce the time required to sterilize the larger samples by 15 to 90 minutes as compared to air dry samples, but had little effect on the time required to sterilize the smaller samples.

4. Sterilizing the samples at 100 per cent of water holding capacity did not appear to have much advantage over sterilizing at 50 per cent of water holding capacity.

5. Steaming under pressure resulted in an increase in the amount of base-exchangeable ammonia in the soils and the peat. This increase varied from 1 to 30 parts per million in the soils and from 18 to 160 parts per million in the peat.

6. The amount of ammonia released by steaming under pressure appeared to be least for the air dry samples despite the more severe treatments required to sterilize such samples.

7. Sterilizing by means of one steam treatment appeared to result in less ammonia increase than sterilizing by shorter consecutive treatments.

8. Steaming under pressure did not appear to result in significant changes in the nitrate content of the fifty gram samples but tended to reduce the amounts present.

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